
Using Synchrotron-based FTIR Microspectroscopy (SFTIRM) to Reveal the Differences of Endosperm Structural and Chemical Make-up among Six Barley Varieties

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Abstract

Barley grains are widely used for malting and feeding purposes in Canada. Although barley varieties have similar chemical composition, they exhibit different rumen degradation characteristics and nutrient availability. The objectives of this study was to determine structural make-up features and identify the structural differences in chemical functional groups in endosperm tissue among the six barley varieties using ultra-spatially resolved synchrotron-based FTIR microspectroscopy (SFTIRM). The results indicated that the barley varieties showed significant differences in terms of peak area intensities and the peak ratios of the amide I (1650 cm^{-1}) and amide II (1550 cm^{-1}), cellulosic compounds (ca. 1240 cm^{-1}), and non-structural carbohydrates (NSC, starch) peak (1025 cm^{-1}). The synchrotron-based FTIR spectroscopic information associated with structural and chemical make-up characteristics of barley grains may provide more information as to why barley varieties exhibit different biodegradation behaviors.

Introduction

As one of the most important annual cereal grains, barley is a main source for malting and serving as animal feed in Canada. Barley varieties exhibit different rumen degradation characteristics and nutrient availability though they have similar chemical composition (Yu et al. 2003). Knowledge of their chemical and structural differences may lead to an understanding of the reasons for these differences. Today, FTIR microspectroscopy coupled with synchrotron radiation is widely used in biological applications to get the visible image and spectral information together and to characterize the microscopic sample area at the cellular or subcellular levels without thermal noise (Wetzel et al. 1998; Dumas and Miller 2003; Marinkovic and Chance 2005; Yu et al. 2007). Accordingly, taking advantage of the brilliance, broadband and concentration (Miller and Dumas 2006), the application of synchrotron light associated with FTIR technique makes it possible to probe further on the inherent structural and chemical make-up of biological materials (Dumas and Miller 2003).

Objectives

The objectives of this study were using synchrotron-based FTIR microspectroscopy to determine structural make-up features and identify the structural differences in chemical functional groups in endosperm tissue among the six barley varieties.

Materials and Methods

Six barley varieties were used in this study, including AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey, and CDC Cowboy. Seeds of each barley variety were randomly selected to cut transversely across the endosperm tissue. The thin cross sections of tissues (ca. 6 μm) were unstained and immediately mounted on BaF_2 discs for synchrotron FTIR microspectroscopic work. Spot samples were randomly selected in endosperm area between 100-600 μm from outside of the seed section. The IR microspectroscopy instrument was coupled with synchrotron radiation from U2B beamline, Brookhaven National Laboratory National Synchrotron Light Source, U.S. Department of Energy (NSLS-BNL, Upton, NY). The spectra were collected through an aperture of $10 \times 10 \mu\text{m}$ in a transmission mode within mid-IR spectral range of ca. 4000-800 cm^{-1} . The spatial resolution was set as 4 cm^{-1} and 128 scans were coadded on each spot to produce an IR spectrum (Yu et al. 2004). Statistical analyses were performed using the MIXED procedure of SAS 9.1.3 (SAS Institute, Inc., Cary, NC). Fisher's protected LSD test was used to compare means with $P < 0.05$ considered significant.

Results and Discussion

Ultra-spatially resolved synchrotron-based FTIR microspectroscopy was used to monitor the intrinsic distribution of chemical compounds associated with nutrition in barley endosperm tissue. The results of this study show that there were significant differences ($P < 0.05$) among the six barley varieties in terms of peak area intensities and the peak ratios of the amide I (1650 cm^{-1}) and amide II (1550 cm^{-1}), cellulosic compounds (ca. 1240 cm^{-1}), and non-structural carbohydrates (NSC, starch) peak (1025 cm^{-1}) (Figure 1).

CDC Helgason exhibited the smallest value (9.19) whereas McLeod showed the greatest intensity (12.59) of Amide I plus II peak area. CDC Trey had the relatively smaller value (0.47) of the peak area at ca. 1240 cm^{-1} (cellulosic compounds band). McLeod had the smallest ($P < 0.05$) NSC peak area (Figure 2). Through application of the synchrotron-based FTIR microspectroscopy, it is possible to characterize and classify the inherent molecular structural features among the different barley varieties.

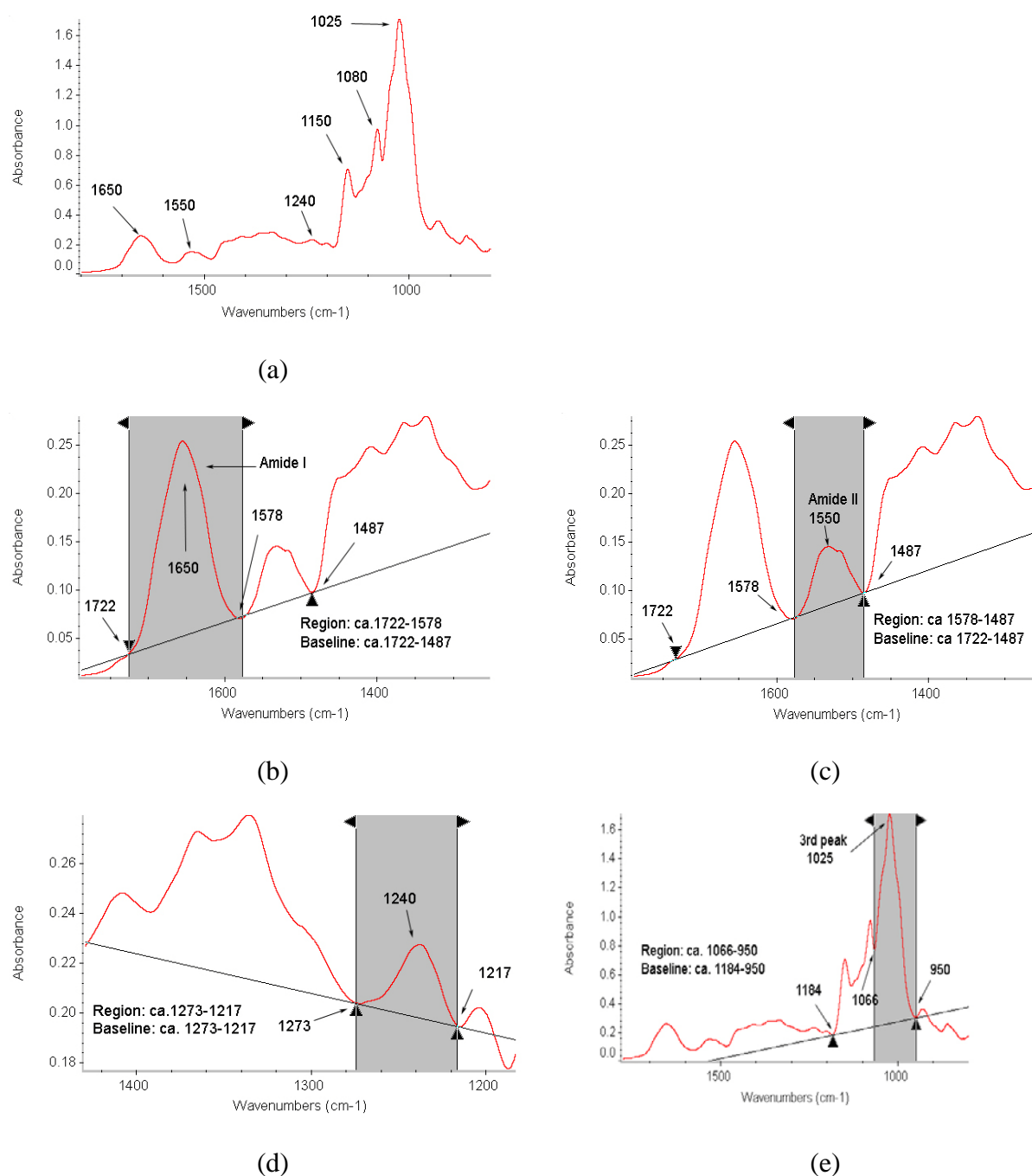


Figure 1. Typical synchrotron-based FTIR spectrum of endosperm tissue within a cellular dimension: (a) fingerprint region: ca. 1800-800 cm^{-1} ; (b) amide I peak area: ca. 1650 cm^{-1} ; (c) amide II peak area: ca. 1550 cm^{-1} ; (d) cellulosic material peak area: ca 1240 cm^{-1} ; (e) non-structural (NSC, starch) carbohydrate peak area: ca. 1025 cm^{-1} .

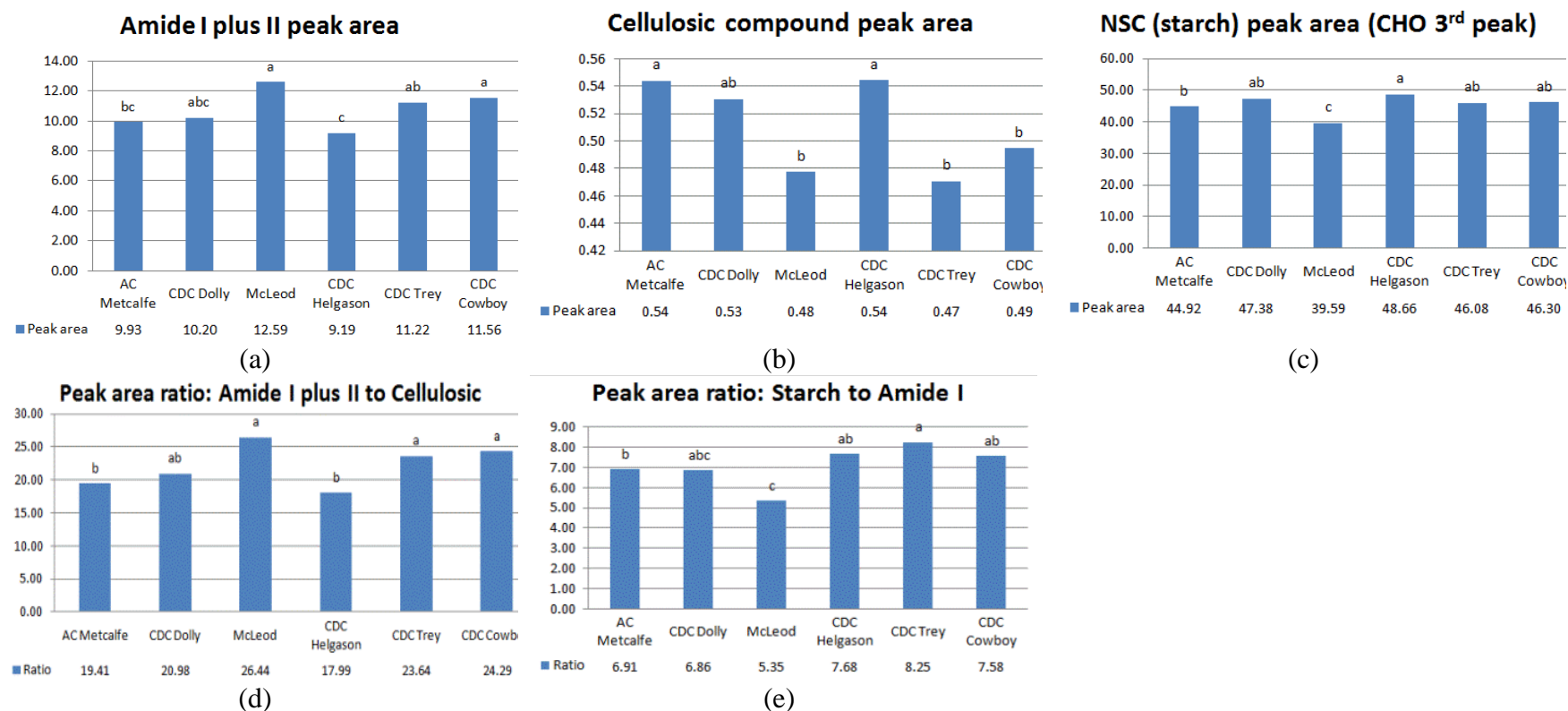


Figure 2. The structural characteristics of protein amide I and II, structural (cellulosic compounds) and non-structural (NSC, starch) carbohydrates (CHO) in the endosperm tissue of barley varieties, revealed using Synchrotron-based FTIR Microspectroscopy: Comparison of six barley varieties. (a) amide I (ca. 1650 cm^{-1}) plus amide II (ca. 1550 cm^{-1}) peak area; (b) cellulosic material peak area: ca 1240 cm^{-1} ; (c) non-structural (NSC, starch) carbohydrate peak area: ca. 1025 cm^{-1} ; (d) the peak area ratios of protein amide I plus amide II to cellulosic compounds; (e) the peak area ratios of non-structural (NSC, starch) carbohydrates to protein amide I.

Conclusion

This study demonstrated that significant variation exists in structural and chemical composition among barley varieties. The ultra-spatially resolved synchrotron based FTIR microspectroscopy (SFTIRM) can be used to reveal the structural and chemical make-up within cellular and subcellular dimensions without chemical degradation of the inherent structure of cereal grain tissue. The structural differences of barley seeds may be one reason for the various digestive behaviours and nutritive values for ruminants. More research is needed to improve these spectroscopic techniques for grain evaluation and cultivar selection.

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